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Concentration of Poliovirus from Tap Water Using Positively Charged Microporus Filters

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Concentration of Poliovirus from Tap Water Using Positively Charged Microporous Filters

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Microporous filters that are more electropositive than the negatively charged filters currently used for virus concentration from water by filter adsorption-elution methods were evaluated for poliovirus recovery from tap water. Zeta Plus filters composed of diatomaceous earth–cellulose–"charge-modified" resin mixtures and having a net positive charge of up to pH 5 to 6 efficiently adsorbed poliovirus from tap water at ambient pH levels 7.0 to 7.5 without added multivalent cation salts. The adsorbed viruses were eluted with glycine-NaOH, pH 9.5 to 11.5. Electropositive asbestos-cellulose filters efficiently adsorbed poliovirus from tap water without added multivalent cation salts between pH 3.5 and 9.0, and the absorbed viruses could be eluted with 3% beef extract, pH 9, but not with pH 9.5 to 11.5 glycine-NaOH. Under water quality conditions in which poliovirus recoveries from large volumes of water were less than 5% with conventional negatively charged filters and standard methods, recoveries with Zeta Plus filters averaged 64 and 22.5% for one- and two-stage concentration procedures, respectively. Electropositive filters appear to offer distinct advantages over conventional negatively charged filters for concentrating enteric viruses from water, and their behavior tends to confirm the importance of electrostatic forces in virus recovery from water by microporous filter adsorption-elution methods.

To determine the public health significance of human enteric viruses in drinking water supplies, reliable, sensitive, and practical methods for detecting small quantities of viruses in large volumes of water must be developed and used. Virus concentration from large volumes of drinking water by adsorption to and subsequent elution from microporous filters is presently considered the most promising method (9, 16), and it has been tentatively adopted as a standard procedure (1). In this method viruses are adsorbed to filters from the flowing water and then recovered by elution with a small volume of fluid. Viruses in the eluate are quantitated by conventional biological assays. Virus adsorption to these filters is reversible and is thought to be at least partly electrostatic in nature (14). Efficient virus adsorption to presently used filters occurs only if the water is acidified to about pH 3.5 (17) and/or multivalent cation salts are added (16). Adsorbed viruses are eluted with slightly basic protein solutions or glycine-NaOH buffers, pH 10.5 to 11.5 (6, 16). Drawbacks of current adsorption-elution methods—including the extensive chemical modification of the water sample, the strongly acidic and basic pH levels utilized, the formation of precipitates during reconcentration, their susceptibility to variations in the quality of the water sample, and their overall complexity, unreliability, and limited usefulness—have prompted this investigation of improvements in virus concentration methodology.

Recent electrophoretic studies have established that the microporous filters now employed for concentrating viruses from water are negatively charged throughout the pH range of 2 to 7, with the net negative charge approaching neutrality below pH 2 (13). Electrophoretic studies of viruses indicate that most viruses are negatively charged near neutral pH and have isoelectric points below pH 7 (3). The fact that both adsorbent filters and viruses are negatively charged near neutral pH may explain the need for acidification or the addition of multivalent cations to obtain efficient virus adsorption from water. Adsorption may be more efficient at pH 3.5 than at pH 7.5 because there is likely to be electrostatic attraction between viruses and filters at the lower pH, whereas there is electrostatic repulsion near neutral pH. Near neutral pH, multivalent cations could serve to mediate the adsorption reaction between the two negatively charged surfaces (4, 5).

Microporous filters that are more positively charged in the pH range of most natural and tap waters (pH 5 to 9) would appear to offer advan-

tages over negatively charged filters as virus adsorbents by reducing or eliminating the need for either acid or salt addition to obtain virus adsorption. Furthermore, if the filters became negatively charged at pH levels somewhat higher than those of tap waters, then it might be possible to elute the adsorbed viruses under moderately alkaline conditions. In this study, presently available positively charged microporous filters were evaluated for concentrating poliovirus from tap water.

(Portions of this paper were presented at the 1978 Annual Conference of the American Water Works Association; Proc. Ann. Conf. Am. Water Works Assoc., in press. Portions of this paper were also presented at the Fourth International Congress for Virology, The Hague, The Netherlands, 30 August–6 September 1978.)

MATERIALS AND METHODS

Virus and virus assay. Plaque-purified poliovirus type 1, strain LSc, was used as a model for the enteroviruses that may be present in water. Viruses were grown and assayed by using Buffalo Green Monkey kidney (BGM) cells as previously described (18); quantitation was by the plaque technique. Purified, monodispersed viruses, prepared by freon extraction and sucrose density gradient centrifugation (8), were used in experiments with low virus levels.

Filter media. Negatively charged filter media had a 0.45-μm porosity and were composed of fiberglass-asbestos (type M-780, series AA, Cox Instrument Div., Lynch Corp., Detroit, Mich.) and fiberglass (no. 8025–035, Filterite Corp., Timonium, Md.). Positively charged media had a 0.5-μm porosity and were composed of asbestos-cellulose (Seitz, grade S, Alsop Engineering Corp., Milldale, Conn.) or had a 0.75- and 0.45-μm porosity (types 50S and 60S, respectively) and were composed of cellulose-diatomaceous earth-"charged-modified" resin (series S, Zeta Plus, AMF Cuno, Meriden, Conn.). All virus-adsorbent filters were used as circular disks in either polypropylene or stainless-steel filter holders. For experiments with large volumes of tap water, 1-μm porosity, 10-inch (ca. 2.54 cm) long polypropylene filter cartridges (model M39R10S, Commercial Filters Div., Carborundum Co., Lebanon, Ind.) were used with Zeta Plus 50S filters to improve flow rates.

Electrophoresis of filter media. The electrophoretic mobility of filter media as a function of pH was determined by Briggs cell microelectrophoresis by using the procedure of Black and Smith (2). Filter media were blended in distilled water for 1 to 5 min and either filtered through gauze or allowed to settle in order to obtain particle suspensions of suitable size for electrophoretic analysis. Suspensions were buffered with 0.001 M acetate at pH 5, phosphate at pH 7, and borate at pH 9. Sodium chloride was added to a final 0.05 M concentration to assure constant ionic strength.

Virus concentration experiments. For virus concentration experiments in which water volumes were 12 liters or less, 47-mm diameter virus-adsorbent filters were used. Volumes of cold Chapel Hill tap water were dechlorinated with 10 mg of sodium thiosulfate per liter. Portions were adjusted to desired pH levels with 1.0 or 0.1 N HCl or NaOH and then seeded with a small volume of stock virus to give a desired virus concentration. Depending upon their volumes, the samples were placed in either 600-ml capacity polycarbonate or 20-liter capacity stainless-steel pressure vessels, and small samples were taken for initial virus assay. The samples were filtered through microporous filters at the desired flow rate by using a regulated nitrogen gas cylinder as a source of positive pressure. The filtrates were collected and assayed for viruses. Adsorbed viruses were recovered from the filters by filtering two successive 7.5 ml-volumes of eluent fluid through the filters and collecting the eluates dropwise. The pH of the collected eluate was quickly measured and then adjusted to pH 7.4 with 0.05 M glycine-hydrochloride, pH 1.5. After measuring the total volume, the eluate was assayed for viruses.

For experiments with 378-liter (100-gal) volumes of water, the apparatus and general procedures described in the tentative standard method were used (1, 10, 11). Virus-adsorbent filters were housed in 267-mm diameter stainless-steel holders, and viruses were added to the flowing tap water along with the sodium thiosulfate solution via one pump of a triplex fluid proportioner (Johanson and Son Machine Corp., Clifton, N.J.). When the tap water was acidified to pH 3.5 before virus adsorption, dilute HCl was added to the flowing tap water by using two alternating pumps of the fluid proportioner.

RESULTS

Electrophoretic mobility of filter media particles as a function of pH. Electrophoretic mobility measurements of various filter media particles were made in solutions ranging in pH from 1.5 to 11.0. The results of these measurements are given in Table 1, and they are also plotted in Fig. 1 along with the Filterite data of Kessick and Wagner (13). The electrophoretic mobility of each type of filter medium became more electropositive with decreasing pH, presumably due to protonation of functional groups. Zeta Plus 50S and 60S were the most electropositive filter media, with isoelectric points between pH 5 and 6. Cox AA was somewhat more

TABLE 1. *Electrophoretic mobility of various filter media*

pH	Electrophoretic mobility (μm/s/V/cm)[a]			
	Filterite	Cox	Zeta Plus 50S	Zeta Plus 60S
1.5	−0.60	+0.70	ND[b]	ND
3.0	−1.80	+0.30	+1.02	+1.10
5.0	−2.40	−1.00	+0.26	+0.46
7.0	−2.60	−2.00	−0.74	−0.52
9.0	ND	ND	−1.70	−1.18
11.0	ND	ND	−1.51	−1.67

[a] Ionic strength, 0.05.
[b] ND, Not done.

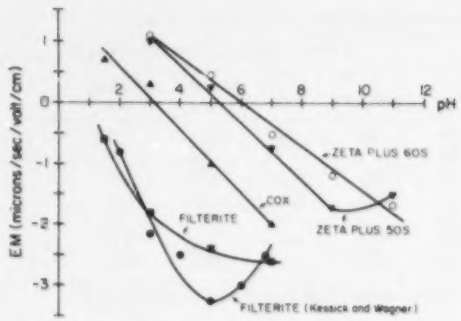

FIG. 1. *Electrophoretic mobility of filter media as a function of pH.*

electronegative with an isoelectric point between pH 3 and 4, while Filterite was the most electronegative with an isoelectric point below pH 1.5. The results for Filterite particles are consistent with those of Kessick and Wagner (13), who found that particles of fiberglass, as well as cellulosic filters, had negative electrophoretic mobilities as low as pH 2. They suggested that filters possessing strongly negative electrophoretic mobilities near neutral pH are predictably poor virus adsorbents in that pH range because the electronegativity of both the filter and virus surfaces would cause them to electrostatically repel each other unless multivalent cations were added to help chemically mediate an adsorption reaction. Because Zeta Plus filters have considerably higher isoelectric points than Cox or Filterite filters and are more electropositive throughout the pH range tested, it is possible that they would adsorb negatively charged viruses at considerably higher pH levels than the electronegative filters without having to add multivalent cations. Cox filters, which are electropositive up to about pH 3.5, are expected to be intermediate between the more electronegative Filterite filters and the more electropositive Zeta Plus filters in terms of their ability to adsorb viruses.

It should be mentioned that measurements of the electrophoretic mobilities of Seitz filter particles were also made. The mobility curve was similar in shape to the Zeta Plus curves, having an isoelectric point near pH 7. However, in view of the fact that chrysotile asbestos particles are isoelectric near pH 12 (15), the measured isoelectric point for the Seitz filters may be low and may be an artifact of the particle preparation procedure. Chrysotile asbestos is a hollow fiber structure with a strongly electropositive nature due to the unique geometrical and spatial characteristics of its charged functional groups. Strongly electropositive magnesium hydroxide functional groups compose the outer, exposed surface of the fiber, whereas the strongly electronegative silicates are confined to inner, unexposed surfaces. A possible explanation for a lower than expected isoelectric point is that the blending required for particle preparation may have sheared the fibers, exposing highly negative silicate groups which were previously unexposed, and thereby lowering the net surface charge of the particles.

Effect of pH on poliovirus adsorption to various filters. As an initial step in evaluating positively charged filters for concentrating viruses from water, the effect of tap water pH on poliovirus adsorption was determined. Volumes of 500 ml of dechlorinated tap water were adjusted to the pH levels shown in Table 2, seeded with about 10,000 plaque-forming units (PFU) of poliovirus per ml and assayed for initial virus concentration. These volumes were filtered through the indicated media at fluxes of about 10 ml per cm^2 per min, and the filtrates were assayed for viruses. Two successive 7.5-ml volumes of pH 11.5 glycine-NaOH were passed through each filter to elute adsorbed viruses. The total 15-ml eluate was quickly adjusted to pH 7.4 and assayed for viruses. Although poliovirus was efficiently adsorbed to the negatively charged Filterite and Cox filters at pH 3.5 and was efficiently eluted with pH 11.5 glycine-NaOH, virus adsorption, and, hence, recovery efficiencies, were greatly reduced at pH 5.5 and 7.5 (Table 2). It should be noted that the more electropositive Cox filters adsorbed approximately twice as much virus at pH 5.5 and 7.5 as the more electronegative Filterite filters. Zeta Plus 50S filters efficiently adsorbed viruses between pH 5.5 and 7.5, but not at the higher or lower pH levels tested, while Zeta Plus 60S filters efficiently adsorbed viruses between pH 3.5 and 6.0, but were somewhat less effective at the higher pH levels tested. Figure 2 clearly shows the superiority of Zeta Plus 50S over Cox and Filterite filters as a virus adsorbent in the pH range of most natural and tap waters. For both Zeta Plus 50S and 60S filters, the majority of adsorbed viruses were recovered by elution with pH 11.5 glycine-NaOH. Seitz filters adsorbed virtually 100% of poliovirus from tap water at pH 3.5 to 9, but the adsorbed viruses could not be eluted with pH 11.5 glycine-NaOH. Because Zeta Plus 50S and 60S and Seitz S filters are capable of efficiently adsorbing polioviruses at considerably higher pH levels than the more electronegative filters, such as Cox and Filterite, they may be capable of adsorbing viruses directly from tap water at ambient pH

TABLE 2. *Effect of tap water pH on poliovirus adsorption to various filter media*[a]

Adsorption pH	% Total input virus adsorbed					% Total input virus recovered in eluate				
	Filterite	Cox	Zeta Plus 50S	Zeta Plus 60S	Seitz	Filterite	Cox	Zeta Plus 50S	Zeta Plus 60S	Seitz
3.5	93	99	63	>99	>99	66	50	38	77	0
5.5	17	34	98	>99	>99	4	9	69	73	0
6.0	ND[b]	ND	ND	>99	ND	ND	ND	ND	60	ND
7.0	ND	ND	99	72	>99	ND	ND	63	45	0
7.5	7	18	99	61	>99	3	8	67	42	0
8.5	ND	ND	26	ND	ND	ND	ND	1	ND	ND
9.0	ND	ND	ND	ND	>99	ND	ND	ND	ND	0

[a] Tap water volume, 500 ml; filter diameter, 47 mm; elution was with pH 11.5 glycine-NaOH, 15 ml.
[b] ND, Not done.

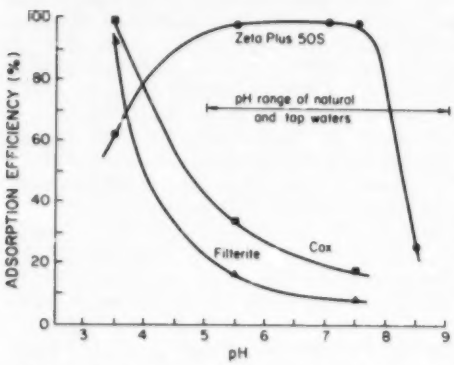

FIG. 2. *Poliovirus adsorption to filter media as a function of pH.*

levels without having to add multivalent cations.

Virus elution from positively charged filters. Positively charged Zeta Plus 50S and 60S and Seitz S filters were further studied with respect to the conditions for eluting adsorbed viruses. Although the previous experiments showed that the majority of viruses adsorbed to both Zeta Plus filter types could be recovered by elution with glycine-NaOH at pH 11.5, these filters were further tested to determine whether efficient virus elution with glycine-NaOH could be obtained at lower pH levels. Elution at pH levels lower than 11.5 is desirable because many enteric viruses are unstable under such extremely alkaline conditions. In these experiments 500-ml volumes of dechlorinated tap water seeded with about 10,000 PFU of poliovirus per ml were filtered through 47-mm diameter Zeta Plus 50S and 60S filters at pH 7.0 and 6.0, respectively, for virus adsorption. These pH levels were selected to assure at least 99% virus adsorption, and the filtrates were assayed for viruses to confirm that this adsorption efficiency had been achieved. The adsorbed viruses were then eluted with two successive 7.5-ml volumes of 0.05 M glycine buffer at either pH 11.5, 10.5, 10.0, or 9.5, and the eluates were quickly adjusted to pH 7.4 and assayed for viruses. As shown by the results in Table 3, greater than 60% poliovirus elution efficiency was obtained at all pH levels tested, including pH 9.5 where virus recoveries averaged 88 and 74% for Zeta Plus 50S and 60S, respectively. Statistical evaluation of these data showed no significant difference in elution efficiency between pH 9.5, 10.5, and 11.5 ($P > 0.10$ in all cases). However, it should be noted that the lowest elution efficiency occurred with pH 11.5 glycine-NaOH, which may reflect some virus inactivation at this extreme pH. The ability to obtain efficient virus elution under moderately alkaline conditions, such as pH 9.5 to 10, minimizes possibilities of virus inactivation by hydroxide alkalinity and allows for longer contact times between filter and eluent.

As previously noted, elution of poliovirus from Seitz filters was not possible using glycine-NaOH at pH 11.5. This would be expected if Seitz filters are as strongly electropositive as believed (isoelectric point at pH 11 to 12) and if elution by strongly basic buffers relies on electrostatic repulsion to overcome the forces of adsorption, because no net charge reversal of the filter would take place at pH 11.5. In further investigations it was found that 3% beef extract at pH 9 was an effective eluent for Seitz filters.

TABLE 3. *Effect of pH on poliovirus elution from Zeta Plus filters with glycine-NaOH*[a]

Filter	Elution efficiency (%) at pH:			
	11.5	10.5	10.0	9.5
50S	65	70	ND[b]	88
60S	67	82	79	74

[a] Tap water volume, 500 ml; filter diameter, 47 mm; elution volume, 15 ml.
[b] ND, Not done.

In these experiments poliovirus was adsorbed to Seitz filters at ambient tap water pH levels (7.0 to 7.5) and eluted with various eluents using the general procedures previously described for the elution experiments with Zeta Plus filters. The results of these experiments (Table 4) indicate that greater than 70% elution efficiency was possible with 3% beef extract at pH 9. Elution efficiency was lower when 3% beef extract was used at pH 7, and the lowest elution efficiencies were obtained with pH 9 and 11.5 glycine-NaOH. This demonstration of effective virus elution from Seitz filters, coupled with their extremely wide range for efficient virus adsorption, further establishes the usefulness of highly electropositive filters in concentrating viruses from tap water at ambient pH levels.

Reconcentration of poliovirus from primary eluates. Reconcentration of viruses in primary eluates obtained by processing 378 liters (100 gallons) of tap water was evaluated for Zeta Plus filters. Primary eluates were obtained by filtering 378 liters of dechlorinated tap water at pH 6 through a 267-mm diameter Zeta Plus 60S filter disk, eluting with 1 liter of glycine at pH 10, and neutralizing to pH 7.4. This primary eluate was then subdivided into appropriate volumes and seeded with poliovirus. Both Zeta Plus 50S and 60S filters were tested for virus reconcentration efficiency at two adsorption pH levels and two elution pH levels, and the results of these experiments are given in Table 5. In the reconcentration step, greater than 90% virus adsorption efficiency was obtained at pH 6 and 5, respectively, for Zeta Plus 50S and 60S. These are only slightly lower than the adsorption pH levels used in primary virus concentration. The slightly lower pH required for virus adsorption in the reconcentration step may be due to the higher organic content of the primary eluates compared to tap water. Some of these organics, particularly humic and fulvic acids, may compete with viruses for adsorption sites, thereby reducing the efficiency of virus retention (7; Sobsey et al., manuscript in preparation). Efficient elution of adsorbed viruses was obtained with pH 10 as well as pH 11.5 glycine-NaOH. Thus, with only moderate pH adjustments and no addition of multivalent cation salts, both Zeta Plus filter media were capable of efficiently reconcentrating polioviruses from primary eluates.

Concentration of low levels of poliovirus using Zeta Plus 50S. Large volume experiments with low input virus levels were done in which 12-liter volumes of tap water were processed through 47-mm diameter Zeta Plus 50S filters as ambient pH levels, ranging from pH 7.0 to 7.5. Processing 12 liters through a 47-mm diameter filter is approximately equivalent in hydraulic loading to processing 378 liters through the 267-mm diameter filters normally employed for large-scale primary virus concentration. Adsorbed viruses were eluted with 15 ml of pH 10 glycine-NaOH, and the entire eluate was assayed for viruses. The mean virus recovery for six experiments was 64%, and the median virus recovery was 56% (Table 6). In a similar experiment with Seitz filters virus recovery was 28% when adsorbed viruses were eluted with 3% beef extract at pH 9.0. It should also be noted that in similar experiments during the same time period using Cox filters under the tentative standard method (1) conditions of adsorption at pH 3.5 and elution with pH 11.5 glycine-NaOH, virus recoveries were consistently less than 5%

TABLE 4. *Poliovirus elution from Seitz S filters*[a]

Eluent	Elution efficiency (%)
3% Beef extract, pH 9	73
3% Beef extract, pH 7	30
Glycine-NaOh, pH 9	1
Glycine-NaOh, pH 11.5	<1

[a] Tap water volume, 500 ml; filter diameter, 47 mm; adsorption at ambient tap water pH (7.0 to 7.5); elution volume, 15 ml.

TABLE 5. *Poliovirus concentration from primary eluate (reconcentration)*[a]

Filter	Adsorption pH	Adsorption efficiency (%)	Recovery efficiency (%)[b]	
			pH 10	pH 11.5
Zeta Plus 50S	7.5	53	75	40
	6.0	98	75	85
Zeta Plus 60S	6.0	85	110	85
	5.0	92	110	110

[a] Primary eluate volume, 500 ml; filter diameter, 47 mm.
[b] Elution with 15 ml of glycine-NaOH.

TABLE 6. *Recovery of low levels of poliovirus from 12-liter volumes of tap water by using Zeta Plus 50S filters*[a]

Expt No.	Amt of virus (PFU)		Vol concn factor	Input virus recovered (%)[b]
	Input	Recovered		
1	22	14	460	64
2	53	30	460	57
3	97	43	460	44
4	217	119	475	55
5	33	44	370	133
6	144	46	480	32

[a] Filter diameter, 47 mm; adsorption at ambient tap water pH (7.0 to 7.5); elution with pH 10.0 glycine-NaOH, 15 ml.
[b] Mean was 64; median was 56.

(Rutala and Sobsey, manuscript in preparation). These poor virus recoveries under tentative standard method conditions are consistently obtained with Chapel Hill tap water during the late fall, winter, and early spring, and they apparently result from interference caused by soluble organic matter in the finished tap water (Sobsey et al., manuscript in preparation). The large difference in virus recovery efficiency between Zeta Plus 50S and Cox filters suggests that Zeta Plus filters may be less susceptible to certain interferences in the water and, therefore, may be more suitable for waters of widely varying quality than conventional electronegative filters.

Large-scale virus concentration with Zeta Plus 50S filters. To demonstrate the applicability of Zeta Plus 50S filters to a more realistic situation involving 378-liter (100-gallon) volumes of tap water, 267-mm diameter filter disks were used in the primary step of a two-stage flow-through adsorption-elution experiment. Low levels of monodispersed poliovirus were seeded by a fluid proportioner into 378-liter volumes of tap water, and concentrated by two-stage filter adsorption-elution procedures to final volumes of about 25 ml. Two Cox filters, one under adsorption-elution conditions identical to those for the Zeta Plus 50S filters and another under conditions specified in the 14th ed. of *Standard Methods* (1), were utilized in simultaneous experiments as controls. Virus recoveries were much greater with the Zeta Plus filters than with either of the Cox filters—20 and 25% recovery with Zeta Plus filters and only 2 and 4% recovery with Cox filters (Table 7). These results further demonstrate the superiority of Zeta Plus filters as virus adsorbents under certain conditions. In addition, the concentration procedure was much simpler when Zeta Plus filters were used because the need for continuously adding controlled amounts of acid and/or salts was eliminated.

DISCUSSION

The optimum conditions for concentrating poliovirus from drinking water by using the positively charged filters investigated in this study are summarized in Table 8. Electropositive Zeta Plus 50S and 60S and Seitz S filters were more effective poliovirus adsorbents than electronegative Cox and Filterite filters in the pH range of most natural and tap waters. The electropositive filters efficiently adsorbed poliovirus from tap water at ambient pH levels ranging from pH 7.0 to 7.5 with no added polyvalent cation salts. By eliminating the need for chemical modifications of the water, expensive fluid proportioners or other elaborate chemical additive systems become unnecessary. Elimination of such complexities may make it possible to obtain more consistent virus recoveries from water.

Adsorbed viruses were efficiently eluted from primary Zeta Plus filters with pH 9.5 to 10.0 glycine-NaOH, and the viruses in the primary eluate were reconcentrated by adsorption to and elution from a second, smaller Zeta Plus filter. Because viruses adsorbed to Seitz asbestos-cellulose filters could be effectively eluted only with 3% beef extract at pH 9.0, reconcentration by adsorption to and elution from filters is not possible, and an alternative reconcentration procedure such as "organic flocculation" (12) be-

TABLE 8. *Optimum conditions for concentrating poliovirus from tap water by using electropositive microporous filters*

Filter type	Adsorption pH	Elution conditions
Zeta Plus 50S	5.5–7.5	Glycine-NaOH, pH ≥ 9.5
Zeta Plus 60S	3.5–6.0	Glycine-NaOH, pH ≥ 9.5
Seitz S	3.5–9.0	3% Beef extract, pH 9.0

TABLE 7. *Comparison of Cox and Zeta Plus filters for two-stage concentration of poliovirus from 378-liter volumes of tap water*

Adsorbent filters[a]	pH conditions				Amt of Virus (PFU)		Virus recovered (%)
	Primary concn		Reconcn				
	Adsorption	Elution	Adsorption	Elution[b]	Input	Recovered	
Zeta Plus 50S	7.1	10	6	10	413	103	25
Zeta Plus 50S	7.1	10	6	10	426	85	20
Cox	7.1	10	6	10	407	8	2
Cox[c]	3.5	11.5	3.5[d]	11.5	305	13	4

[a] Primary and secondary adsorbents were 267- and 47-mm diameter, respectively. Zeta Plus primary adsorbent was preceded by a 1-μm porosity polypropylene prefilter.

[b] Elution with 0.05 M glycine-NaOH.

[c] Conditions specified in *Standard Methods*, 14th ed. (1).

[d] With 0.0005 M $AlCl_3$.

comes necessary. Because adsorbed polioviruses are effectively recovered from the electropositive filters tested by using only mildly alkaline eluents, the opportunities for virus inactivation are reduced and virus recoveries may be improved. It is possible that positively charged filters will be effective for concentrating those viruses that have been found to be unstable under the highly alkaline elution pH conditions of the tentative standard method (1), such as adenoviruses, reoviruses, rotaviruses, and parvoviruses (Sobsey et al., manuscript in preparation). The use of less alkaline eluents also results in a greater degree of volume concentration because less glycine-hydrochloride is needed to neutralize the eluates.

The results of this study indicate that positively charged filters may be less susceptible to certain chemical interferences in drinking waters because they effectively concentrated polioviruses under water quality conditions in which the negatively charged filters of the tentative standard method gave poor recoveries. Another advantage of positively charged filters related to water quality is the elimination of precipitation and resultant filter clogging caused by acidifying or adding multivalent cation salts to primary eluates. Although the results of this study indicate distinct advantages of positively charged filters over conventional negatively charged filters for virus concentration from tap water, additional studies are needed to determine whether these positively charged filters are suitable for other types of waters.

The results of positively charged filters tend to support the hypothesis that electrostatic forces are important in virus adsorption to and elution from microporous filters. The differences in the electrostatic characteristics of various filter media may at least partially explain their observed differences with respect to virus adsorption and elution. Virus adsorption occurs most efficiently under pH conditions where the net charges on the virus particles and the filter media are either opposite in sign or small in magnitude (that is, near their isoelectric points). Under pH conditions which result in sizable net charges of like sign, virus adsorption is considerably less efficient, and the adsorption efficiency decreases as the magnitudes of the charges increase. Glycine elution of adsorbed viruses is efficient under pH conditions where both the virus particles and the filters have sizable net charges of like sign, presumably because of strong electrostatic repulsive forces. Because electrostatic forces appear to play an integral role in virus adsorption to and elution from microporous filters, these forces should be further studied in more detail in order to better understand and further improve filter adsorption-elution methods for concentrating enteric viruses from water.

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